UNIVERSITY OF MARYLAND
AGRICULTURAL EXPERIMENT STATION

Etiology and Control of Walnut Anthracnose

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TABLE OF CONTENTS

Contribution No. 3197 of the Maryland Agricultural Experiment Station, Department of Botany

ETIOLOGY AND CONTROL OF WALNUT ANTHRACNOSE[1]

Frederick H. Berry[2]

INTRODUCTION

Walnuts, *Juglans* spp. L., which were numerous and widely distributed during past geological periods, now comprise only about 12 to 15 species (12). They may be divided into three groups: (1) the east Asian walnuts, which are grown very little in the United States; (2) the Persian, or English, species *J. regia* L., which is extensively cultivated in California and Oregon; and, (3) the North American walnuts, of which there are several species, the most important being the eastern black walnut, *J. nigra* L. Butternut, *J. cinerea* L., or white walnut, is also a North American member of the genus *Juglans* (1).

The eastern black walnut is found over a wide area. Its natural range extends from central New England to Georgia in the east and from Minnesota to Texas in the west. It attains its best development in the Ohio River Valley, but it is common at practically all elevations in the eastern United States up to about 1,400 feet, where it is replaced by butternut (12). The eastern black walnut is highly prized for both its lumber and fruits.

Walnut anthracnose, or leaf blotch as it is sometimes called, is a widespread and destructive disease of certain walnut species, particularly the eastern black walnut. It is caused by a fungus, *Gnomonia leptostyla* (Fr.) Ces. & de N., of which the imperfect stage is *Marssonina juglandis* (Lib.) Magn. When climatic conditions are very favorable, this disease may quickly become epidemic. Consequently, many black walnut trees are almost entirely defoliated by late July or early August. This premature defoliation often results in poorly-filled, low-quality nuts. The disease also reduces growth, greatly weakens the trees, and in some cases contributes to their early death.

Walnut anthracnose is world-wide in distribution. Besides infecting *Juglans* species in the United States, the disease has been reported from most of the countries of Europe (2,8,15), Argentina (11), Canada (5), and South Africa (9).

A relatively small amount of research has been done on walnut anthracnose. Klebahn (13), a German mycologist, investigated the disease in the early 1900's, and Carter (3) and Carter and Hoffman (4) conducted some experiments at the University of Illinois between 1943 and 1952 to ascertain methods for control. To obtain further information on the nature of the organism causing walnut anthracnose and its host relations and to devise ways of controlling it, the writer began the present studies in 1955.

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[1]Adapted from a thesis submitted to the Graduate School, University of Maryland, in partial fulfilment for the degree of Doctor of Philosophy. Grateful appreciation is extended to Dr. Carroll E. Cox, of the University of Maryland, for guidance in these studies and especially for aid in preparation of the manuscript; to Dr. Harley L. Crane, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture and Mr. G. F. Gravatt, formerly with Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, for their advice during progress of the work; and to Mr. C. D. Schlemmer, Fredericksburg, Virginia, for the use of his orchard for some of the spraying experiments.

[2]Formerly Plant Pathologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland, now Forest Pathologist, Forest Service, United States Department of Agriculture, Columbia, Missouri.

[3]Figures in parentheses refer to literature cited, page 22.

MATERIALS AND GENERAL METHODS

The development and control of the disease were studied under a natural environment in the walnut orchards at the Plant Industry Station, Beltsville, Maryland, and in the C. D. Schlemmer orchard near Fredericksburg, Virginia. Plant materials used in the greenhouse and in laboratory studies were obtained from either the orchards or the nursery at Beltsville.

At Beltsville, the effectiveness of various protective fungicides in controlling walnut anthracnose was tested in a 3½-acre orchard containing 28 eastern black walnut trees approximately 30 years old. These trees are of 6 different varieties, Rohwer, Ohio, Stambaugh, Stabler, Thomas, and Ten Eyck. Fungicides were applied with a hydraulic sprayer that developed approximately 600 pounds of pressure at the spray gun. From 20-40 gallons of spray mixture was applied to each tree depending on its size. Spray applications included either 25 percent wettable parathion (1 pound to 100 gallons of water) and 50 percent wettable DDT (4 pounds to 100 gallons of water) or 25 percent wettable malathion (2 pounds to 100 gallons of water) for insect control. Certain trees serving as controls were sprayed with water and insecticides only.

Also at Beltsville, the effectiveness of eradicant ground sprays for control of anthracnose was tested in 1956 and 1957. In 1956 the experiments were carried on in the Cherry Hill orchard using a conventional high-pressure hydraulic sprayer to apply the spray materials while the following year the 3½-acre experimental orchard was used and the ground spray was applied with a conventional hydraulic sprayer equipped with a horizontal-type, multinozzle **boom.**

At Fredericksburg, the number of applications of a protective fungicide necessary to control walnut anthracnose was studied. The orchard consisted of approximately 500 bearing black walnut trees, most of which were of the Stabler variety; eighty-six trees of the Stabler variety were used in the study.

The experimental design consisted of 3 randomized blocks with each of 5 treatments occurring once in each block. The experimental design was as follows:

Treatment	*No. of zineb sprays*	*No. of plots*	*No. of trees*
A	4	3	20
B	3	3	18
C	2	3	18
D	1	3	16
E	0	3	14

The trees were sprayed with zineb (Dithane Z-78), a fungicide which had given effective control of walnut anthracnose during previous experimental work. The fungicide was applied at the rate of 6 pounds per 100 gallons of water with an axial-flow, air-blast sprayer having a tank capacity of 50 gallons. This sprayer used turbulent air to atomize and apply concentrated sprays. In this experiment a round air nozzle with four 1/8 inch mist-type liquid nozzles mounted in the center of the air stream was used, and the liquid was under a pressure of approximately 30 pounds per square inch.

Approximately 4 to 4½ gallons of this concentrated spray was applied to each tree. Spray applications included 25 percent wettable malathion at the rate of 2 pounds per 100 gallons of water for control of insects, particularly lace bugs (*Corythucha* sp.).

For estimation of severity of anthracnose infection, 100 leaflets were chosen at random from each tree. A 10-foot ladder was used so that leaflets might

be obtained high up in the crown of the trees, as well as from the lower branches. The number of leaflets showing necrotic spots caused by the fungus indicated the percentage of infection for each tree.

In addition to the percent of leaflets infected, each tree was rated for premature defoliation. This defoliation rating was as follows: none, 0; very light, 1; light, 2; considerable, 3; heavy, 4; very heavy, 5; complete, 6.

RESULTS AND DISCUSSION

The Pathogen

Ascogenous stage: Gnomonia leptostyla, an ascomycete, is a member of the family Gnomoniaceae. Large numbers of perithecia of this fungus were observed in the spring in overwintered walnut leaves infected the previous season by the anthracnose organism. The perithecia measured 200-300 microns in diameter, just as reported by Klebahn (13). The asci were 50-70 microns long and 8-14 microns thick. In the latitude of Beltsville, Maryland, asci reached maturity from the middle to the latter part of May. The 8 hyaline, spindle-shaped ascospores in each ascus lay mostly in 2 layers, each consisting of 4 spores. On reaching mature size the spores were 2.5-4.0 microns thick and 18.0-25.0 microns long. They were divided into 2 cells of equal size by a septum.

Discharge of ascospores was followed under field conditions by placing glass slides, coated on the lower side with glycerine jelly about 5 millimeters above walnut leaves which bore abundant ascocarps of *G. leptostyla.* The glass slides were examined and changed daily. Ascospores, which cause primary infection, were discharged only during rainy periods and were apparently carried to walnut leaves by wind or a combination of wind and rain. The discharge period varied with seasonal conditions, but usually most of the ascospores were discharged by early June. The foliage on most black walnut varieties was from one-half to three-fourths mature size at the time of ascospore discharge. During 1955-1959 the earliest observed occurrences of the disease at Beltsville, Maryland, were as follows: June 13, 1955; May 31, 1956; June 6, 1957; May 23, 1958; and May 25, 1959. Lesions appeared first on the underside of the leaflets, possibly because in the opening of the bud the lower surface of the leaflet was more exposed and subject to wetting.

Conidial stage: Black subepidermal acervuli of *Marssonina juglandis* were observed on the lesions shortly after primary infection was first visible. The acervuli were 100-200 microns in diameter and were frequently arranged in concentric circles. They were much more abundant on the underside of the leaflets; but occasionally a few were found on the upper side. The conidia were 3-4 microns thick and 14-30 microns long, slightly thicker than the 2-3 microns reported by Klebahn (13). They were colorless, usually crescent-shaped, pointed above, truncate below, and divided by a septum into 2 cells of approximately the same size.

In addition to the typical conidia of *M. juglandis,* unicellular, bacillar spores measuring 1-2 x 6-12 microns were sometimes found in an acervulus. Klebahn (13) also observed these spores and believed them to be involved in the developmental process of *M. juglandis.* Wolf and Wolf (16) reported that *G. leptostyla* produced bacillar spermatia in acervuli concurrently with conidia of *M. juglandis.*

The time at which dissemination of conidia, which cause secondary in-

fection, took place was determined by exposing microscope slides coated with glycerine jelly in an orchard where anthracnose was present. No conidia were caught during dry weather, but during rainy weather large numbers of conidia were found in droplets of water on the slides.

Pathogen-Host Relations

Symptoms: The fungus attacked the leaves, nuts, and occasionally the shoots of the current season's growth. The necrotic leaf spots were 1/16 to 5/16 inch in diameter, dark brown, and more or less circular in shape (Fig. 1). Often several spots coalesced and formed larger necrotic areas. The leaf tissues bordering these spots were usually a yellowish to golden color.

In July 1956, 50 leaflets were collected at random from a black walnut tree and the number and size of lesions per leaflet were determined. There were 1 to 36 lesions per leaflet, averaging 7.9 lesions per leaflet. The lesions were from pinpoint size to 8 millimeters in diameter, averaging about 1.6 millimeters.

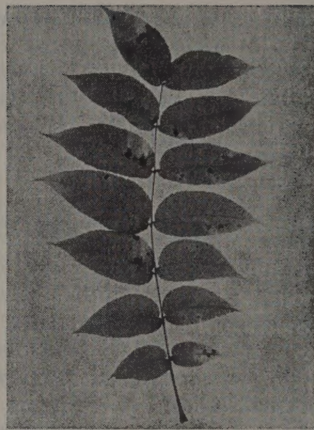

Fig. 1. Anthracnose lesions on a black walnut leaf from tree in orchard at Plant Industry Station, Beltsville, Maryland, 1956.

Premature defoliation generally followed infection but in some instances infected leaflets remained attached to the tree for most of the season. There did not appear to be any correlation

Fig.2. Fallen black walnut leaflets with few to many anthracnose lesions, Beltsville, Maryland, 1955.

between the number of lesions on a leaflet and defoliation. Leaflets under a diseased black walnut tree had few to many lesions (Fig. 2).

The fungus caused sunken, necrotic spots on the husks of the nuts. These lesions were generally smaller than those on the leaves and were circular to irregularly circular in outline (Fig. 3). If infection occurred while the nut was very young, the latter did not develop normally and often dropped prematurely.

Lesions on the stems of the current season's growth were reported by Miller et al. (14). They were oval to irregularly circular, sunken, light grayish-brown dead areas with dark reddish-brown margins.

Overwintering of the fungus: The fungus overwinters primarily in fallen walnut leaves infected during the preceding summer. In these diseased leaves, the fungus develops an ascigerous stage which produces ascospores in the spring. Since Miller et al. (14) reported that the fungus also overwintered in the conidial stage in lesions on twigs of infected walnut trees, the writer examined several hundred twigs from infected black walnut trees during the dormant season. In only two instances, however, was the anthracnose fungus found. On October 28, 1955, conidia of *M. juglandis* were found in acervuli on a leaf scar of the preceding year's growth (Fig. 4). In April 1956, acervuli were noted on a leaf scar on a black walnut twig. The twig was held overnight in a moist chamber and subsequent microscopic examination revealed conidia with the size and appearance of those of *M. juglandis*. The fungus was also found to overwinter occasionally in old infected nuts on the ground, but generally over-

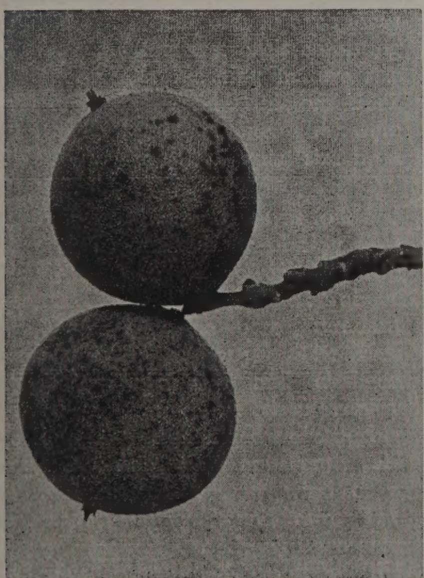

Fig. 3. Anthracnose lesions on nuts of black walnut from tree in orchard at Plant Industry Station, Beltsville, Maryland, 1958.

Fig. 4. Acervuli of *M. juglandis* on leaf scar of black walnut (X 12).

wintering in plant parts other than the fallen leaves was rare.

Artificial inoculation: In the spring of 1958 nursery-grown black walnut and Persian walnut seedlings were transplanted to 10-inch pots. Part of the potted seedlings were brought into the greenhouse and the remainder were placed in a cold frame. On June 12, 3 of the black walnut seedlings and 3 of the Persian walnut seedlings in the cold frame were inoculated by atomizing the leaflets with a freshly prepared suspension of conidia of *M. juglandis* obtained from black walnut leaves. Three additional plants of each of the 2 species were similarly treated with sterile distilled water as a control. Polyethylene bags were placed over each seedling immediately after treatment and left in place for 16 hours, after which the bags were removed. Necrotic lesions caused by the fungus began appearing on all the inoculated black walnut seedlings 16 days after inoculation. Persian walnut seedlings were not infected and no lesions developed on uninoculated controls.

The experiment was repeated with 3 additional seedlings of each of the 2 species on June 16. The polyethylene bags remained over the seedlings for 18 hours. Necrotic lesions began showing up on the black walnut seedlings 14 days after inoculation. Again neither the Persian walnut nor the control seedlings were infected.

On August 1, 3 black walnut seedlings and 3 Persian walnut seedlings growing in the greenhouse were inoculated with a spore suspension of *M. juglandis* from black walnut leaves. Three additional plants of each of the 2 species were sprayed with sterile distilled water. The plants were then placed on a greenhouse bench, where they were kept constantly wet by means of an intermittent misting device. Necrotic lesions appeared on the 3 inoculated black walnut seedlings 16 days after inoculation. When the plants were removed on October 8, practically all the leaflets on the inoculated black walnut plants were infected, but no lesions were present on the Persian walnut or control seedlings.

These experiments indicated that under controlled conditions about 14-16 days elapse from the time of inoculation until the first necrotic lesions are visible. Lack of infection on Persian walnut seedlings inoculated with a spore suspension of the fungus from black walnut leaves indicated the possibility that a separate strain of the fungus attacks Persian walnuts.

Interrelation of rainfall and infection: During the 1956 field season the development of anthracnose on 6 unsprayed black walnut trees was followed at Beltsville. Just as the leaves were unfolding, 10 leaves representatively spaced around the crown of the tree on each of the 6 trees were tagged. There were 10 to 23 leaflets on each leaf. The leaflets were examined beginning June 1, but the first lesion was not observed until July 16. Thereafter, lesions on each leaflet were counted at approximately 2-day intervals until defoliation occurred or until the lesions became too numerous to be counted accurately (Table 1).

The first noticeable increase in average number of lesions per leaflet occurred on August 6, approximately 16 days after a heavy rainfall of 1.88 inches. On August 22 there was another noticeable increase in average number of lesions per leaflet, apparently the result of a rainy period on August 5 and 6. However, both of these increases were relatively small as compared with the increase of over 2 lesions per leaflet on September 7. This increase was 17 days after a rainfall of 1.10 inches. On October 1 there was

Table 1.—Relation of rainfall to anthracnose infection of black walnut at Beltsville, Maryland, 1956.

Date	*Number leaflets examined*	*Total number lesions*	*Average no. of lesions/ leaflet*	*Increase in lesions/ leaflet*	*Rainfall*	
					Date	*Amount*
July					July	inches
					18	.15
16	932	1	.001		21	1.88
					22	.03
23	926	2	.002	.001	23	.38
					24	.09
25	923	3	.003	.001	25	.07
27	922	7	.008	.005	27	.11
30	920	7	.008	none		
August						
1	918	10	.011	.003		
3	909	16	.018	.007	August	
					5	.11
6	869	36	.041	.023	6	.08
8	857	65	.076	.035		
10	842	80	.095	.019		
13	829	99	.119	.024		
					14	.04
15	821	110	.134	.015	15	.01
17	816	118	.145	.011	20	.03
20	795	127	.160	.015	21	1.10
22	768	155	.202	.042	22	.05
24	751	173	.230	.028		
27	745	197	.264	.034	27	.04
29	738	195	.264	none		
31	685	170	.248	none		
Sept.						
3	654	229	.350	.102		
5	645	678	1.051	.701	Sept.	
					6	.24
7	618	2022	3.272	2.221	7	.02
10	586	2067	3.527	.255		
12	573	2005	3.500	none		
14	527	1630	3.093	none		

Table 1.—Relation of rainfall to anthracnose infection of black walnut at Beltsville, Maryland, 1956. (Continued)

Date	*Number leaflets examined*	*Total number lesions*	*Average no. of lesions/ leaflet*	*Increase in lesions/ leaflet*	*Rainfall*	
					Date	*Amount*
Sept. 17	397	1076	2.710	none	16	.38
19	350	945	2.700	none	19	.14
21	331	965	2.915	.215	20	.33
					24	.25
25	268	619	2.310	none	27	.46
					28	1.30
Oct. 1	178	731	4.107	1.797	29	.02

another increase of almost 2 lesions per leaflet, 0.38 inch of rain having fallen 15 days previously. In all instances, a sizeable increase in number of lesions per leaflet appeared to be associated with a previous heavy rain.

Species susceptibility: Walnut anthracnose was most destructive on eastern black walnut. Butternut, Persian walnut, and first-generation hybrids of Persian and black walnut were also attacked by the fungus. Gard (10) reported that two species of walnut native to California, the Hinds walnut, *J. hindsii* Jeps., and the California walnut, *J. californica* S. Wats., were both susceptible to anthracnose. Although the foliage of Persian and hybrid walnut trees was often severely infected with anthracnose during wet seasons, considerably less defoliation occurred than on eastern black walnut. Also, lesions on Persian and hybrid walnut leaves tended to be smaller, about 2 millimeters in diameter, and more nearly circular than lesions on black walnut leaves.

Varietal susceptibility: Approximately 40 different varieties are represented among the 100 or more bearing black walnut trees growing in the orchards at Beltsville. When climatic conditions were favorable for anthracnose development, highly susceptible varieties showed practically 100 percent infection of their leaflets in mid-July and complete defoliation followed in August. Even the less susceptible varieties were heavily infected and defoliated in years when anthracnose development was severe.

Each September from 1955 through 1959 the black walnut trees growing in the orchards at Beltsville were examined and their susceptibility to anthracnose recorded.

Following is a summary of the average susceptibility of the various varieties for this period as determined by the procedure outlined on page 2.

Variety	*Defoliation rating*	*Infection (percent)*
Baker	4	93
Creitz	4	89
Depew	4	81
Graham	4	84
Grundy	4	77
Marton	4	75
McMillen	4	92
Pierce	4	91
Roberts	4	81
Sellman	4	86
Shimmoler	4	96
Speer	4	74
Ten Eyck	4	86
Wetzel	4	89
Wiard	4	81
Asbury	3	78
Booth	3	84
Hopwood	3	71
Lamb	3	75
Metcalf	3	76
Monterey	3	71
Myers	3	71
Schwartz	3	82
Stambaugh	3	76
Tastrite	3	80
Thomas	3	78
Throp	3	70
Wanda	3	86
Beck	2	76
Edras	2	84
Franklin	2	66
Germaine	2	67
Hare	2	69
Ohio	2	36
Riehl	2	76
Rohwer	2	36
Sparrow	2	49
Stabler	2	35
Stover	2	62
Todd	2	63
Worthington	2	68

In 1957 it was brought to the writer's attention that black walnut trees of 2 varieties growing in Illinois appeared very resistant to anthracnose. Scionwood was obtained from these varieties, Captain and Victoria, and topworked in a black walnut tree at Beltsville in the spring of 1958. On September 25, 1959, defoliation and infection were as follows:

Variety	*Defoliation rating*	*Infection (percent)*
Captain	2	70
Victoria	2	79

Black walnut varieties may not be equally susceptible to anthracnose in all sections of the country. There is no evidence as to the cause of this variation in susceptibility. It is probably the result of one or more of the following: (1) different geographic races or physiologic strains of *G. leptostyla;* (2) climatic effects on the pathogen; and (3) effect of the environment on the host-parasite relation.

Experimental Control of Anthracnose in the Field[4]

The standard recommendation for control of walnut anthracnose has usually been two or three sprays of bordeaux mixture. Another method of control is to destroy the fallen leaves that harbor the causal fungus. However, results with this method have been erratic, since the overwintering ascocarps are so small that sufficient numbers may be left behind to cause primary infection.

From 1943-47, Carter (3) tested 16 fungicides for the control of walnut anthracnose. He obtained the most effective control in 1946. The trees were given two applications of fungicide on June 17 and July 9. Trees sprayed with Puratized Agricultural Spray 1:5,000 had the fewest leaf spots as well as the least defoliation. Leaf spots were somewhat more numerous on the trees sprayed with Puratized Agricultural Spray 1:10,000 and defoliation was greater.

[4]To simplify presentation in this paper, trade names of pesticides are sometimes used. No endorsement of the named products and no criticism of similar untested products by the U. S. Department of Agriculture are intended.

In 1951 Carter and Hoffman (4) tested 6 fungicides for control of walnut anthracnose. Applications were made on June 13 and July 3. Again, the walnut trees sprayed with Puratized Agricultural Spray at the rate of 1 pint to 100 gallons of water had the fewest leaf spots. These trees averaged 1.1 spots per leaflet as compared with 20.3 for the unsprayed trees.

Experiments with protective fungicides at Beltsville, Maryland: The fungicides tested in 1955 were zineb (Dithane Z-78), bordeaux mixture, glyodin (Crag Fruit Fungicide 341), and glyodin plus phenylmercury acetate (Phix). Sprays were applied on May 12 and 23 and June 2 and 13.

Walnut anthracnose first appeared in this orchard at Beltsville early in July; however, during this month only the Stambaugh variety was severely infected. Between August 9 and September 6, over 12 inches of rain fell and infection greatly increased. The effectiveness of the various fungicides in controlling walnut anthracnose is shown in Table 2.

In general, zineb, an organic zinc compound, was the most effective fungicide against anthracnose. On four of the walnut varieties—Rohwer, Ohio, Stambaugh, and Stabler—zineb gave the best control; on Ten Eyck and Thomas, bordeaux mixture was the most effective fungicide. Bordeaux mixture also gave fairly good control on the other varieties except Stambaugh. Glyodin and glyodin plus phenylmercury acetate controlled the disease adequately on the Ohio variety, but gave poor control on the other varieties. Glyodin plus phenylmercury acetate gave slightly better control than glyodin by itself.

During 1956 the two fungicides that proved most effective against anthracnose in 1955, zineb and bordeaux mixture, together with phenylmercury triethanol ammonium lactate (Puratized Agricultural Spray) and ziram (Zerlate) were used in an attempt to control anthracnose. The trees were sprayed 4 times, May 19 and 29 and June 8 and 27.

Although in another orchard at Beltsville the Creitz variety was infected with anthracnose on May 31, the disease was not observed in this experimental orchard until July 16, on leaflets of an untreated check tree of the Ten Eyck variety. Rainfall was very light (4.83 inches) between July 1 and Sep-

Table 2.—Effectiveness of various protective fungicides in control of walnut anthracnose, Beltsville, Maryland, 1955.

Materials	*Amount of fungicide in 100 gallons of water*	*Number of trees treated*	*Infection of leaves on Sept. 6*[1]	*Estimated defoliation on Sept. 6*
			percent	percent
Zineb	2 lb.	5	21	14
Bordeaux mixture	6 lb. copper sulphate, 2 lb. lime	6	32	16
Glyodin + phenylmercury acetate	1 pt. + 2 oz.	3	50	28
Glyodin	1 qt.	6	59	33
None (control)[2]	----	8	97	53

[1]Average infection based on 100 leaflets per tree.
[2]Sprayed with water and insecticides.

Table 3.—Effectiveness of various protective fungicides in control of walnut anthracnose, Beltsville, Maryland, 1956.

Materials	*Amount of fungicide in 100 gallons of water*	*Number of trees treated*	*Infection of leaves on Sept. 14*[1]	*Estimated defoliation on Sept. 14*
			percent	percent
Zineb	2 lb.	6	1.3	2
Bordeaux mixture	6 lb. copper sulphate, 2 lb. lime	5	1.4	2
Phenylmercury triethanol ammonium lactate	1 pt.	6	2.0	2
Ziram	2 lb.	3	5.0	3
None (control)[2]	----	8	38.5	16

[1]Average infection based on 100 leaflets per tree.
[2]Sprayed with water and insecticides.

tember 15 and the disease developed very slowly. In fact, on August 15 no disease was evident on any of the sprayed trees and infection on the untreated trees was very light. The effectiveness of the various fungicides in controlling walnut anthracnose, on the basis of final disease counts, is shown in Table 3.

It may be seen in Table 3 that walnut anthracnose infection in this orchard was at a relatively low level in 1956. Foliage on most walnut trees remained in fairly good condition until the nuts reached maturity, about the first of October. All the fungicides gave good control of walnut anthracnose. In fact, disease development was so sparse that even the untreated trees were only lightly defoliated.

Beginning about the middle of July, some of the trees were rather heavily defoliated by the walnut caterpillar, *Datana integerrima* G. & R. Also, the population of lace bugs, *Corythucha* sp., became fairly large during the latter part of July and in August and caused the leaves on some of the trees to turn yellow and drop prematurely. Defoliation caused by insects was taken into consideration in estimating defoliation due to anthracnose.

Weather favorable for anthracnose development prevailed throughout the 1958 growing season, and consequently infection was severe. A rain gauge at the orchard indicated the following precipitation: June 3.96 inches, July 7.58 inches, August 5.59 inches, and September 1.26 inches.

Table 4.—Effectiveness of various protective fungicides in control of walnut anthracnose, Beltsville, Maryland, 1958.

Fungicide[1]	*Number of trees treated*	*Infection of leaves on Sept. 11*[2]	*Estimated defoliation on Sept. 11*
		percent	percent
Zineb	5	7.2	5
Maneb	6	8.2	5
Captan	6	70.2	40
Ferbam	6	78.0	50
None (control)[3]	5	99.0	87

[1]All fungicides applied at the rate of 2 pounds per 100 gallons of water.
[2]Average infection based on 100 leaflets per tree.
[3]Sprayed with water and insecticides.

The fungicides tested in 1958 were zineb, which had given good control previously, maneb (Dithane M-22), captan (Captan 50W), and ferbam (Fermate). Fungicides were applied on May 27, June 10, June 24, and July 8 at the rate of 2 pounds per 100 gallons of water. The effectiveness of these fungicides in controlling walnut anthracnose under apparently favorable climatic conditions for development of the disease is shown in Table 4.

Zineb again proved to be the most effective fungicide against anthracnose. Maneb, an organic manganese fungicide, was also very effective. In fact, on 3 of the 6 varieties, maneb gave as good control as zineb or better. Defoliation of trees sprayed with zineb and maneb was negligible (Figs. 5 and 6). Neither captan nor ferbam was effective in controlling anthracnose. Both infection and defoliation were severe on trees sprayed with these two fungicides. Almost 100 percent of the leaflets on the untreated trees were affected with anthracnose. One tree of the Stambaugh variety was entirely defoliated, and over three-fourths of the foliage on all untreated trees was gone by Sept. 11, 1958 (Fig. 7).

Further trials were conducted in 1959 to compare the relative efficiencies of 4 different fungicides in the control of walnut anthracnose. In addition to maneb and zineb, two new fungicides, dodine (Cyprex) and phaltan, were used. The trees were sprayed on May 27, June 11, June 26, and July 16. The final spray was about 6 days late, because of inclement weather.

The 1959 growing season was characterized by irregular rainfall and high temperatures. A rain gauge maintained at the orchard indicated the following precipitation: June, 2.46 inches; July, 4.50 inches; August, 2.15 inches; and, September, 1.21 inches. Secondary infection occurred during suitable moist periods, particularly in July.

Fig. 5. Black walnut, Stabler variety, which received 4 applications of zineb. Photographed at Beltsville, Maryland, September 11, 1958.

Fig. 6. Black walnut, Ohio variety, which received 4 applications of maneb. Photographed at Beltsville, Maryland, September 11, 1958.

Fig. 7. Black walnut, Ohio variety, check tree (unsprayed). Photographed at Beltsville, Maryland, September 11, 1958.

Table 5.—Effectiveness of various protective fungicides in control of walnut anthracnose, Beltsville, Maryland, 1959.

Fungicide	*Amount of fungicide per 100 gallons of water*	*Number of trees treated*	*Infection of leaves on Sept. 17*[1]	*Estimated defoliation on Sept. 17*
	pounds		percent	percent
Zineb	2	5	3.6	5
Maneb	2	6	5.2	5
Dodine	1	6	5.2	5
Phaltan	2	6	14.8	20
None (control)[2]	....	5	77.2	82

[1]Average infection based on 100 leaflets per tree.
[2]Sprayed with water and insecticides.

The effectiveness of the 4 fungicides in controlling walnut anthracnose under these climatic conditions is shown in Table 5.

Zineb was again most effective in controlling walnut anthracnose. Maneb and dodine were only slightly less effective. Phaltan did not give satisfactory control of the disease. The unsprayed trees were severely infected and defoliated.

Experiments with eradicant fungicides at Beltsville, Maryland: The walnut anthracnose fungus overwinters primarily in fallen walnut leaves infected during the preceding summer. Ascospores ejected through the openings of perithecia buried in the tissues of these dead walnut leaves cause primary infection in the spring.

In 1956, it was decided to investigate the possibility of a control program based on the use of eradicant sprays alone and in conjunction with protective sprays. Monocalcium arsenite (Corono CM-220) and phenylmercury triethanol ammonium lactate (Puratized Agricultural Spray), two fungicides which had previously proved satisfactory as dormant, eradicant fungicides for pecan scab (6,7), were used in a field test at Beltsville, Maryland. The trees, representing a number of different varieties of the eastern black walnut, had all been severely infected with anthracnose the previous summer.

Four plots, each consisting of 6 trees, were established. In each of the plots receiving spray treatment, 3 of the trees were sprayed with monocalcium arsenite at the rate of 3 pounds per 100 gallons of water, and 3 of the trees were sprayed with phenylmercury triethanol ammonium lactate at the rate of 5 pints per 100 gallons of water. The spray treatments were as follows:

Plot 1 — 1 dormant eradicant spray
Plot 2 — 2 dormant eradicant sprays
Plot 3 — none (control)
Plot 4 — 2 dormant eradicant sprays plus 4 summer protective sprays of zineb at the rate of 2 pounds/100 gallons of water.

The first eradicant spray was applied on April 21, 1956, when both the trees and the ground beneath them to 10 feet beyond the edge of limb spread were sprayed. The second eradicant spray was applied on April 27. Approximately 45 gallons of the spray material was applied to each tree and the ground around it. The four summer protective sprays were applied on May 19 and 29 and on June 8 and 27. During the summer, data were taken monthly on the development of walnut anthracnose on these trees. These data are summarized in Table 6.

Table 6.—Effectiveness of various fungicides in control of walnut anthracnose, Beltsville, Maryland, 1956.

Plot	Number of trees treated	Fungicide	Leaves infected on		
			July 16	*Aug. 15*	*Sept. 20*
			percent	percent	percent
1	3	Monocalcium arsenite	0	3	60
1	3	Phenylmercury triethanol ammonium lactate	0.7	5	60
2	3	Monocalcium arsenite	0	4	74
2	3	Phenylmercury triethanol ammonium lactate	0	4	39
3	6	None (control)	0.2	4	63
4	3	Monocalcium arsenite plus zineb	0	0	1
4	3	Phenylmercury triethanol ammonium lactate plus zineb	0	0	5

These results indicated that eradicant sprays did not eliminate the need of subsequent protective sprays to control the disease. Although a great deal of the inoculum in the form of ascospores in the diseased leaves was probably destroyed by the eradicants, the amount that escaped the ground treatment was sufficient to cause primary infection. Thereafter, the fungus was able to propagate itself asexually.

In the treatment in which the 2 dormant sprays was followed by the regular summer protective sprays, disease incidence was very low. It is impossible to say whether the dormant sprays contributed materially to control of the disease in this plot, since in this particular test no trees received summer protective sprays alone. However, in another walnut orchard about a mile away, only 2% of the leaves on trees that received 4 summer protective sprays were diseased. This seems to indicate that the dormant eradicant sprays had little or no effect on control of walnut anthracnose.

During the 1957 field season an experiment was set up in the walnut orchard at the Plant Industry Station, Beltsville, Maryland, to test the effectiveness of an eradicant ground spray in reducing the number of subsequent applications of protective fungicides required to control walnut anthracnose. On June 7, 1957, the entire orchard floor was sprayed with Elgetol, a commercial preparation containing approximately 30% of sodium dinitro-orthocresol. Ascospore development was advanced at this time, and a large percentage of the asci were full of mature ascospores. Elgetol in a 0.5-percent concentration by volume was applied at the rate of 400 gallons per acre. On July 24, 1957, the foliage of 14 of the black walnut trees in the orchard was sprayed with a protective fungicide (zineb) at the rate of 2 pounds per 100 gallons of water. The remaining 14 trees did not receive the protective spray treatment.

Infection was very light on all trees in the experiment during the first part of the season. As the season advanced, environmental conditions became favorable for secondary infection and there

Table 7.—Incidence of walnut anthracnose following application of fungicides to ground only and ground plus tree.

Black walnut variety	*Ground only*			*Ground plus tree*		
	No. trees	*Infection*[1]	*Defoliation rating*	*No. trees*	*Infection*[1]	*Defoliation rating*
		percent			percent	
Rohwer	2	23.5	2	2	3.5	1
Ohio	3	30.7	2	4	5.5	1
Stambaugh	3	62.7	3	2	8.0	1
Stabler	2	78.5	3	2	4.0	1
Thomas	2	98.5	4	1	43.0	3
Ten Eyck	2	51.5	3	3	6.7	1

[1]Average infection based on 100 leaflets per tree.

was considerable spread of the disease. On September 13, 1957, records were taken on infection and defoliation. The effectiveness of the two treatments in controlling walnut anthracnose is shown in Table 7.

Walnut anthracnose was prevalent on trees when only the ground was sprayed, but the disease was of little consequence, except on the Thomas variety, when a single protective spray also was applied to the foliage. This experiment indicated that the use of an eradicant fungicide on the orchard floor at the time of ascospore discharge does not eliminate the need of subsequent foliage sprays to control the disease. However, a great deal of the ascospore inoculum in the overwintered leaves is eliminated by the eradicant fungicide. Therefore, it appears that the number of protective sprays applied later in the season can be materially reduced. Under the environmental conditions at Beltsville, Maryland, in 1957, the ground spray plus a single protective spray adequately controlled anthracnose on all the trees in the experiment except those of the Thomas variety. Normally four protective sprays would be applied for anthracnose control.

Spray omission tests at Fredericksburg, Virginia: During the 1957-59 field seasons the number of applications of a protective fungicide necessary to control walnut anthracnose was investigated at the C. D. Schlemmer black walnut orchard, near Fredericksburg, Virginia. Yields of nuts from trees receiving the various spray treatments were determined and nut samples analyzed.

As indicated previously, the occurrence of walnut anthracnose is greatly influenced by temperature and moisture conditions during late spring and summer. In 1957, less than 1 inch of rain fell in this orchard during July, and most of the walnut trees were only lightly infected. However, in August over 4 inches of rain fell and secondary infection increased rapidly. When records on infection and defoliation were taken on September 4 and 6, infection was sufficiently abundant to indicate clearly the effectiveness of the various treatments. Results of this season's work in controlling walnut anthracnose are shown in Table 8.

Results reported in Table 8 indicated that degree of control of infection and defoliation were directly proportional to the number of zineb sprays that were

Table 8.—Effectiveness of various zineb treatments in control of walnut anthracnose, Fredericksburg, Virginia, 1957.

Treatment	*No. of trees treated*	*Infection of leaves*[1]	*Defoliation rating*[2]
		percent	
4 sprays on May 29, June 12, 26, and July 10	20	8.6	0.9
3 sprays on June 12, 26 and July 10	18	27.3	1.7
2 sprays on June 26 and July 10	18	40.0	2.1
1 spray on July 10	16	63.7	2.8
None (control)	14	76.7	3.3
LSD @ 5%	....	14.3	0.7
LSD @ 1%	....	20.8	1.0

[1]Average infection based on 100 leaflets per tree.
[2]Average defoliation rating for the trees receiving the various treatments.

applied. Most effective control of the disease was obtained when the trees received four applications of zineb. These trees also showed the least defoliation. Defoliation of trees that received 3 applications of the fungicide was relatively light, but infection was 27% as compared with 9% for trees receiving four sprays. Trees sprayed with 2 applications of zineb had 40% of the leaves affected with anthracnose and approximately 64% of the leaves on the trees that received only 1 application were affected. Defoliation was correspondingly greater on trees that received these last two treatments. Over three-fourths of the foliage on the unsprayed walnut trees was affected with anthracnose and defoliation was severe.

The spraying experiments, begun during 1957 at the C. D. Schlemmer orchard, were continued in 1958. Walnut anthracnose was much more prevalent in this orchard in 1958 than in 1957. The 1958 growing season was characterized by abundant moisture. A rain gauge was maintained at the orchard and the total rainfall each month from June through September was as follows: June, 5.03 inches; July, 2.74 inches; August, 8.19 inches; and September, 1.60 inches.

In early September foliage on all the unsprayed walnut trees was 100% infected with anthracnose and defoliation was very heavy. Records on the incidence of anthracnose infection and degree of defoliation were taken on September 4 and 5 and are shown in Table 9.

Results reported in Table 9 confirm those from the 1957 field season that degree of infection and defoliation are proportional to the number of applications of fungicide. Leaflets on the trees that received four applications of zineb were 18% infected, which was twice the infection recorded on these trees in 1957. The increased infection, however, was due to the severe development of anthracnose. Infection rose to over 80% on trees that received three applications of zineb as compared with 27% on the same trees in 1957. Foliage on trees that received only one or two applications of zineb was heavily infected and defoliation was severe. The unsprayed walnut trees were 100% infected and very heavily defoliated.

Spraying experiments at the C. D. Schlemmer orchard were continued in 1959. A rain gauge maintained at the orchard indicated the following precipitation: June 3.43 inches; July 3.40

Table 9.—Effectiveness of various zineb treatments in control of walnut anthracnose, Fredericksburg, Virginia, 1958.

Treatment	*No. of trees treated*	*Infection of leaves*[1]	*Defoliation rating*[2]
		percent	
4 sprays on May 28, June 11, 25, and July 14	20	18.1	1.0
3 sprays on June 11, 25, and July 14	18	80.3	3.0
2 sprays on June 25 and July 14	18	93.8	3.6
1 spray on July 14	16	98.3	4.7
None (control)	14	100.0	5.0
LSD @ 5%	----	5.0	0.4
LSD @ 1%	----	7.3	0.6

[1]Average infection based on 100 leaflets per tree.
[2]Average defoliation rating for the trees receiving the various treatments.

inches; August 5.31 inches and September 3.88 inches.

Records on the incidence of anthracnose infection and degree of defoliation caused by the fungus were taken on September 22, 1959. The effectiveness of the various fungicidal treatments in controlling walnut anthracnose is shown in Table 10.

The results reported in Table 10 confirm those obtained in 1957 and 1958. To control anthracnose during seasons favorable to its development, it was necessary to spray the trees four times with a protective fungicide. To protect the foliage as thoroughly as possible it was necessary to spray the trees before primary infection took place. Omission of the first spray resulted in a great increase in the severity of infection and defoliation.

Effect of anthracnose control on quality and quantity of nuts: To determine the effect of anthracnose on the filling of the walnuts and the appearance of the kernels, samples of 100 nuts each were collected from each of 4 trees of the Ohio variety used in the 1958 spraying experiment at Beltsville, Maryland. One of the 4 trees was untreated (control). The other trees received 4 applications of zineb, maneb, or captan.

Table 10.—Effectiveness of various zineb treatments in control of walnut anthracnose, Fredericksburg, Virginia, 1959.

Treatment	*No. of trees treated*	*Infection of leaves*[1]	*Defoliation rating*[2]
		percent	
4 sprays on May 25, June 10, 23, and July 7	20	13.3	1.0
3 sprays on June 10, 23 and July 7	18	53.7	2.7
2 sprays on June 23 and July 7	18	72.0	3.6
1 spray on July 7	16	94.0	4.8
None (control)	14	100.0	5.0
LSD @ 5%	----	18.7	0.6
LSD @ 1%	----	27.2	0.8

[1]Average infection based on 100 leaflets per tree.
[2]Average defoliation rating for the trees receiving the various treatments.

Table 11.—Effect of anthracnose infection on black walnuts produced by Ohio variety, Beltsville, Maryland, 1958.

Fungicide	*Infection of leaflets on Sept. 11*	*Kernel percentage*	*Filling rating*[1]	*Pellicle-color rating*[2]	*Specific gravity of nuts*
	percent				
Zineb	4	29.7	1	1	0.944
Maneb	6	29.6	1	2	0.933
Captan	72	28.9	2	2	0.926
Control	100	25.9	3	3	0.859

[1]1—kernels plump; 2—less than 10% of kernels shriveled; 3—more than 10% of kernels shriveled.
[2]1—light; 2—moderately dark; 3—dark.

Kernel percentage and specific gravity of nuts were used as measures of filling. After the nuts were uniformly cured their specific gravity was determined in the following manner. A wire cage with a door was suspended by a wire and then weighed by a spring scale while immersed in a container of water up to a certain point on the wire. One hundred nuts were weighed and placed in the cage and the cage with the nuts inside was again immersed to the same depth as before, and the weight reading was taken again. Because of the bouyancy of the nuts in water, the cage with the walnuts weighed less than the empty cage and the difference in the two weights represented the bouyancy. The weight of the walnuts divided by the sum of their weight plus their bouyancy gave the specific gravity. After the specific gravity of a sample was obtained, the nuts were cracked and the kernel percentage determined by dividing the weight of the kernels by the total weight of the nuts.

The effect of anthracnose infection on the filling and quality of the nuts produced is shown in Table 11.

A comparison of the data in Table 11 indicated that anthracnose had a pronounced effect on the filling and quality of black walnuts. Specific gravity and kernel percentage of the nuts decreased as anthracnose infection increased. Nut kernels from the unsprayed tree were dark and unattractive and many were shriveled; kernels in the sample from the tree sprayed with zineb were plump and light in color.

In a study at Fredericksburg, Virginia, however, no differences in the quality and filling of nuts of the Stabler variety were apparent as a result of defoliation by the anthracnose fungus. One hundred nuts were collected at random from each of the 15 plots in the experiment in October 1957 and October 1959. No nuts were collected in 1958 because that crop was very light. After the nuts were husked and dried, they were brought into the laboratory and the kernel percentage and in 1959 the specific gravity of the nuts were determined as shown in Table 12.

The analyses of variance for kernel percentage showed no significant difference between treatments. An analysis of variance of the 1959 specific gravities, however, showed a linear response to increasing number of zineb sprays, which was significant at the 5% level. The Stabler variety bore such light crops of nuts during the time this experiment was in progress that infection and premature defoliation by the anthracnose fungus apparently did not affect the quality or filling of the nuts to any appreciable degree.

Table 12.— Effect of anthracnose infection on black walnuts produced by Stabler variety, Fredericksburg, Virginia.

Treatment	Kernel percentage		Filling rating[1]		Pellicle color rating[2]		Specific gravity of nuts
	1957	1959	1957	1959	1957	1959	1959
4 zineb sprays	23.1	23.4	1	1	1	1	0.951
3 zineb sprays	24.8	24.9	1	1	1	1	0.958
2 zineb sprays	26.5	24.3	1	1	1	1	0.949
1 zineb spray	25.1	22.9	1	1	1	1	0.931
Control	24.7	24.1	1	1	1	1	0.930
LSD @ 5%	ns	ns					ns

[1] 1—kernels plump; 2—less than 10% of kernels shriveled; 3—more than 10% of kernels shriveled.
[2] 1—light; 2—moderately dark; 3—dark.

Table 13.—Effect of various zineb treatments on yields of black walnuts at Fredericksburg, Virginia.

Treatment	No. trees	Crop rating		
		1957	1958	1959
4 sprays	20	2.1	1.3	2.4
3 sprays	18	2.1	1.1	2.3
2 sprays	18	2.2	0.9	2.4
1 spray	16	2.6	0.9	2.2
None (control)	14	2.6	0.8	2.1
LSD @ 5%		ns	ns	ns

From 1957-59 the nut crop on each walnut tree in the spraying experiment at Fredericksburg was estimated in October. This was a visual appraisal using the following criteria as a guide:

Yield of nuts	*Crop rating*
None	0
Very light	1
Light	2
Considerable	3
Heavy	4
Very heavy	5

The yield for trees in the various treatments is shown in Table 13.

It will be noted that in Table 13 the crop-rating figure is carried to one decimal place. For example in 1957 the 20 trees that received 4 zineb sprays were given a crop rating of 2.1. In this case, 2 of the trees were given a rating of 1; 14 were given a rating of 2; and 4 were given a rating of 3. Therefore, the mean for the 20 trees was 2.1. The data on crop yields were analyzed but were not found to be significant in relation to treatments the trees received.

SUMMARY

The nature of *Gnomonia leptostyla,* the fungus causing walnut anthracnose, its host relations, and methods of controlling the disease were investigated.

The fungus overwintered primarily in fallen walnut leaves infected with anthracnose the previous season. Primary infection was from ascospores, which were discharged from the overwintered walnut leaves only during rainy periods and carried to walnut foliage by wind or a combination of wind and rain. Conidia, which cause secondary infection, were disseminated only during rainy weather.

Inoculation of potted black walnut seedlings indicated that under controlled conditions about 14-16 days elapse from the time of inoculation until the first necrotic lesions are visible.

Walnut anthracnose was most destructive on eastern black walnut. Butternut, Persian walnut, and first-generation hybrids of Persian and black walnut were also attacked by the fungus. Varieties of eastern black walnut varied in their susceptibility to anthracnose. However, when climatic conditions were favorable for anthracnose development even the less susceptible varieties were severely infected and defoliated.

In spraying experiments over a 5-year period zineb (Dithane Z-78) gave consistent and effective control of anthracnose. It gave adequate control of the disease in 1958 when anthracnose was most severe. Other fungicides tested for a shorter period that appeared to give satisfactory control were bordeaux mixture, phenylmercury triethanol ammonium lactate (Puratized Agricultural Spray), maneb (Dithane M-22), and dodine (Cyprex). Other fungicides tested did not give satisfactory control of anthracnose under the climatic conditions encountered in the experiments. None of the fungicides caused any injury to the walnut foliage.

Experiments in which certain spray applications were omitted showed that 4 applications of a protective fungicide were necessary to control anthracnose. To protect the trees from primary infection it was necessary to apply the first spray before ascospore discharge took place. Omission of the first spray application resulted in a great increase in the severity of infection and defoliation.

Nut samples taken from trees of the Ohio variety showing varying degrees of premature defoliation showed that as defoliation increased, specific gravity and kernel percentage of the nuts decreased. Nuts from trees on which the disease was controlled contained plump, light-colored kernels. On other trees as defoliation increased the percentage of nuts having dark, unattractive, shriveled kernels increased. There was no significant difference in the specific gravity or kernel percentage of nut samples from trees of the Stabler variety in various stages of premature defoliation. Apparently this variety bore such light crops of nuts during the period the orchard was under study that premature defoliation caused by the anthracnose fungus did not affect the quality or filling of the nuts.

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